REMARKS

Entry of this response and reconsideration of the above-referenced application is respectfully requested. Reconsideration and withdrawal of the rejections set forth in the Office Action dated April 28, 2005 are respectfully requested. Applicants petition the Commissioner for a 3-month extension of time. A separate petition accompanies this response.

I. Rejections under 35 U.S.C. §103

A. The Present Claims

Applicant's invention, as embodied by independent claim 1, is directed to a method of electrophoretically injecting a sample containing multiple charged components into a microfluidic device, and electrophoretically separating the components. The method as claimed employs a two-electrode injection step and a two-electrode separation step, and combines this voltage control scheme with ITP stacking, by supplying in either the sample solution or the buffer electrolyte solution a high mobility ion which is present at a higher concentration than the sample ions. This method produces detectable peaks of high sharpness and resolution, while maintaining high sample volume, thus producing high peak intensities as well.

As discussed further below, cited reference Manz *et al.* describes a "pushback" strategy (which is referred to in the Applicants' specification as "pullback"), and cited reference Ramsey *et al.* describes a "pinch" injection strategy. Both of these schemes employ simultaneous control of at least three electrodes. They have been shown to increase sample resolution and signal-to-noise ratio, but usually at the expense of signal intensity, due to loss of sample.

Applicants have found that the use of transient isotachophoretic (ITP) stacking, which can be implemented as recited in steps (a)-(c) of claim 1, employed in combination with a two-electrode injection and two-electrode separation strategy (i.e. sample and drain voltage on for injection, followed by upstream and downstream voltage on for separation), gave significantly better peak sharpness and intensity than a "pinch/pullback" injection scheme, when the latter was done either with or without such

ITP stacking. This is illustrated in Fig. 5C, which superimposes Figs. 5A and 5B. As shown in the Figures, while the "pinch/pullback" strategy gives a sharp and well resolved signal peak, its intensity is greatly reduced relative to the "floating" strategy, presumably due to sample loss.

The top two traces in Fig. 6A (which are offset for clarity) compare the "pushback" strategy described in Manz *et al.* (labeled "FL INJ, PB SEP"; no pinching, no stacking) with the method of the invention (labeled "FL + STACK"; i.e. two electrode injection and two electrode separation, with ITP stacking). These results show that the method of the invention clearly provides greater peak sharpness and much greater peak intensity, with near-equivalent resolution (i.e. peak separation).

B. Claims 1, 4-7 and 14-24 were rejected under 35 U.S.C. §103(a), as being unpatentable over Manz *et al.* (U.S. Patent No. 6,280,589) in view of Krivankova *et al.* (*J. Chromatography B*, **689**:13-34, 1997). These rejections are respectfully traversed in light of the following remarks.

1. The Cited Art

Manz *et al.* describes, in the Background section and at column 5, lines 31-58, a two-electrode sample injection process, in which sample is introduced into a main channel by applying an appropriate voltage to each of two channels intersecting the main channel, one of which contains sample. A problem with this process, as described by Manz *et al.* (e.g. at column 1, lines 51-67 and column 5, lines 59-63), is leakage of residual sample from the side channel(s) into the main channel after sample introduction is formally completed.

To address this problem, Manz *et al.* describes the "pushback" (aka "pullback") strategy, where voltage is applied to induce flow back into the side channels, to prevent the above-described leakage (column 5, line 63 to column 6, line 5; column 6, lines 20-39).

As described in the Applicants' specification, this strategy improves resolution and signal-to-noise ratio, but it also leads to loss of sample volume.

Krivankova *et al.* describes the phenomenon of "sample induced stacking" or "sample induced transient ITP". It illustrates a coupled system where transition occurs from an ITP stacking mode to a CZE mode. The systems described employ capillaries, not microfluidic channels.

2. Analysis

a. No Motivation to Combine References

The Applicants contend that one skilled in the art would not have been motivated to combine the teachings of Krivankova *et al.* with those of Manz *et al.* The two references describe systems having significant structural differences and uses, and are directed to different perceived problems and benefits.

Manz *et al.* describe a system in which sample is electrokinetically introduced into a main separation channel region between side channels in a "double T" configuration, as illustrated in Fig. 3 of the patent. In using this "valveless device", "injection of the sample plug into the electrolyte channel is accomplished electro-kinetically by applying an electric field across the supply and drain channels" (column 2, lines 60-63). In order to avoid the resulting problem of bias of sample composition, due to different electrophoretic mobilities of the components (noted at column 2, lines 8-12), the injection is carried out "for a time at least long enough that the sample component having the lowest electrophoretic mobility is contained within the geometrically defined volume" (column 2, lines 63-66).

It can be seen, therefore, that this system employs fairly small sample volumes, since a representative aliquot of the original sample must be electrokinetically introduced into the "geometrically defined volume" between the two side channels.

Krivankova *et al.* describe the usefulness of preceding capillary zone electrophoresis (CZE) with a preconcentration step employing capillary isotachophoresis (ITP). The advantage of this combination, as described, for example, at page 15 of the reference, is that, due to the preconcentrating effect of ITP, "a larger volume of a more diluted sample could be injected", allowing detection of analytes present at low concentration in a sample. In order to handle the "relatively large volume of a sample", a "capillary of wide diameter

equipped with a sample valve is recommended" for injection (page 18, second column). The ITP and CZE capillaries are "interconnected via a bifurcation block which ensures that only a proper part of the ITP zone stack is transferred into the CZE step", concurrent with "removal of the ballast from the sample" (page 19).

Accordingly, Krivankova *et al.* teaches the use of a sample valve, for injecting a large volume of sample, and a "bifurcation block" for transferring a small portion of this volume, following ITP, to the separation zone.

This injection process clearly differs significantly from that shown in Manz *et al.* There is no provision in the device of Manz *et al.* to allow for these large sample volumes, or for "bifurcation", to remove "ballast" sample volume, as taught in Krivankova *et al.*

The references are also directed towards different perceived problems and solutions. In Krivankova *et al.*, as noted above, the problem is the analysis of samples having analytes present in low concentration, such that large sample volumes are required for accurate detection (as described at page 15, second column). This is addressed by pre-concentrating the sample by ITP and diverting the "ballast" solution prior to CZE separation.

Manz *et al.*, on the other hand, is directed to solving the problem of leakage of residual sample from the side channel(s) of their "valveless injection device" into the main separation channel after sample introduction is formally completed (as described at column 1, lines 51-67 and the paragraph bridging columns 5-6). This problem is addressed by applying voltage, during separation, to induce flow back into the side channels, a strategy referred to as "pushback" (aka "pullback").

b. Unsuggested Benefits

One way for a patent applicant to rebut a prima facie case of obviousness is to make a showing of "unexpected results," i.e. to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected. *In re Soni*, 54 F.3d 746, 34 USPQ2d 1684 (Fed. Cir. 1995).

As described in Section A above, the method of the present claims gave significantly better peak sharpness and intensity than a "pinch/pullback" injection

scheme, when the latter was done either with or without such ITP stacking.

There is no suggestion in either of the above-cited references that the use of ITP stacking in a two-electrode ("floating") injection/separation scheme would address the "leakage" problem noted by Manz *et al.*, in a microchannel device, providing high peak sharpness and good resolution, without the concomitant loss of signal intensity that results from the Manz *et al.* method (described in Applicants' specification as "pullback").

The conclusion that the significantly better peak sharpness and intensity is not unsuggested (Office action mailed April 28, 2006) is simply untrue. None of the prior art references teaches that such significantly improved results are possible. As noted above, the method of Manz *et al.* improves resolution, but leads to loss of sample volume and therefore cannot provide the advantages of the present method. Krivankova *et al.* does not even address the problems associated with microfluidic separation. Nor would one skilled in the art expect that these significant improvements be possible based on the state of the art. Therefore, the significantly improved peak sharpness and intensity is absolutely unsuggested.

Nor is the assertion that there is no demonstration that the significantly improved peak sharpness and intensity is the result of the claimed method correct. As described on page 14, line 9 through page 15, line 8, the same microfluidic device, solutions, samples, and reagents were used to compare the method of Manz *et al.* with the presently claimed method. Therefore, the unsuggested results obtained with the claimed voltage scheme must be attributed to the method.

In view of the foregoing, Applicants respectfully request the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

C. Claims 1, 4-7, and 14-24 were rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Shultz-Lockyear *et al.* in view of Krivankova *et al.* The rejections are respectfully traversed in light of the following remarks.

1. The Cited Art

SHULTZ-LOCKYEAR *ET AL.* relate to the effects of injector geometry and sample matrix

on sample loading of an electrophoretic device.

KRIVANKOVA *ET AL.* is described above.

2. Analysis

One skilled in the art would not be motivated to combine the cited references along the lines of the present method as the two references describe systems having significant structural differences and uses, and are directed to different perceived problems and benefits. Similar to the Manz *et al.* reference, Shultz-Lockyear *et al.* describes a system in which sample is electrokinetically introduced into a main separation channel region between side channels in a "double T" configuration. Injection of the sample plug into the electrolyte channel is accomplished electro-kinetically by applying an electric field across the supply and drain channels. Krivankova *et al.*, on the other hand, describe the usefulness of preceding capillary zone electrophoresis (CZE) with a preconcentration step employing capillary isotachophoresis (ITP).

This injection process clearly differs significantly from that shown in Manz *et al.* There is no provision in the device of Manz *et al.* to allow for these large sample volumes, or for "bifurcation", to remove "ballast" sample volume, as taught in Krivankova *et al.*

The references are also directed towards different perceived problems and solutions. In Krivankova *et al.*, as noted above, the problem is the analysis of samples having analytes present in low concentration, such that large sample volumes are required for accurate detection (as described at page 15, second column). This is addressed by pre-concentrating the sample by ITP and diverting the "ballast" solution prior to CZE separation.

Shultz-Lockyear *et al.*, on the other hand, is directed to investigating the effects of injector geometry and solution ionic strength on injection and sample loading. On page 537, col. 2, Shultz-Lockyear *et al.* describes many parameters that affect the "complex behavior" for the "double-T" injector design.

Nor does the combination of Shultz-Lockyear *et al.* and Krivankova *et al.* address the unsuggested results obtained with the present method as described further above and detailed in Figs. 5C and 6A.

Accordingly, Applicants respectfully request the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

D. Dependent claims 11-12 were rejected under 35 U.S.C. §103(a) as being unpatentable over Manz *et al.* in view of Krivankova *et al.*, as applied to parent claim 1 above, and further in view of Ramsey (U.S. Patent No. 6,342,142). The rejections are respectfully traversed in light of the following remarks.

1. The Cited Art

MANZ *ET AL.*, is discussed above.

KRIVANKOVA *ET AL.*, is discussed above.

RAMSEY relates to a microchip apparatus and method for fluidic manipulations.

2. Analysis

Dependent claims 11-12 are directed to types of analytes that may be used in the method of claim 1.

For the reasons discussed above, Manz *et al.* in view of Krivankova *et al.* does not teach or suggest the method of claim 1, or the benefits thereof. With respect to the subject matter of independent claim 1, Ramsey *et al.* does not make up for the deficiencies of this combination of references. For example, at column 5, line to 52 to column 6, line 13, Ramsey *et al.* describes the benefits of "pinched" injection over "floating" injection. The reference does not suggest the approach taken by the applicants, where ITP stacking is used in combination with "floating" injection and separation, and provides superior results to the "pinch/pullback" combination.

Accordingly, claim 1 and its dependent claims are nonobvious over this combination of references.

In view of the foregoing, Applicants respectfully request the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

E. Claims 11-12 were rejected under 35 U.S.C. §103(a) as being unpatentable over Shultz-Lockyear *et al.* in view of Krivankova *et al.*, and further in view of Ramsey. The rejections are respectfully traversed in light of the following remarks.

1. The Cited Art

SHULTZ-LOCKYEAR *ET AL.* is discussed above.

KRIVANKOVA *ET AL.* is discussed above.

RAMSEY is discussed above.

2. Analysis

For the reasons discussed above, Shultz-Lockyear *et al.* in view of Krivankova *et al.* does not teach or suggest the method of claim 1, or the benefits thereof. With respect to the subject matter of independent claim 1, Ramsey *et al.* does not make up for the deficiencies of this combination of references. For example, at column 5, line to 52 to column 6, line 13, Ramsey *et al.* describes the benefits of "pinched" injection over "floating" injection. The reference does not suggest the approach taken by the applicants, where ITP stacking is used in combination with "floating" injection and separation, and provides superior results to the "pinch/pullback" combination.

Accordingly, claim 1 and its dependent claims are nonobvious over this combination of references. Therefore, Applicants respectfully request the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

F. Claim 13 was rejected under 35 U.S.C. §103(a) as being unpatentable over Manz *et al.* and Krivankova *et al.* in view of Fuchs *et al.* (U.S. Patent No. 5,630,924). The rejections are respectfully traversed in light of the following remarks.

1. The Cited Art

MANZ *ET AL.* is discussed above.

KRIVANKOVA *ET AL.* is discussed above.

FUCHS *ET AL.* is directed to an assay method in which an analyte binds with a first binding partner, which is labeled, and with a second binding partner, which is modified to impart a significant charge. The binding partners are typically modified antibodies.

The method is stated to improve over previous assays which did not employ the second binding partner, and where electrophoretic separation of unbound analyte from bound complex was less effective. (See, for example, column 1, lines 45-59 and column 2, lines 44-58.)

While there is a general description of electrophoretic mobility of species in Fuchs (column 16, lines 15-52), there is no description of particular voltage-controlled injection schemes, and, in fact, injection may be carried out using a pump rather than by voltage control (column 21, lines 26-38).

One stage of the assay in Fuchs involves mixing of the analyte and binding partners. One way of mixing these components is noted at column 23, lines 51-53, where "the elements of the mixture were concentrated in an electric field using a technique such as isoelectric focusing or isotachophoresis". There is no other reference to isotachophoresis (ITP) in the patent.

2. Analysis

Fuchs *et al.* does not suggest the advantages of ITP in a separation method. As stated above, the only reference to ITP in Fuchs is as one way of mixing the assay components: "the elements of the mixture were concentrated in an electric field using a technique such as isoelectric focusing or isotachophoresis". Presumably, the assay components are "concentrated" to facilitate reaction (binding) before the separation and detection of bound complex is carried out. Mixing of the components is further described at column 14, line 34-41:

> In one embodiment, first binding partner and second binding partner are combined with a sample to produce a mixture in which, if analyte is present, a three-membered complex forms. As used herein, the term "combine" is intended to mean any process by which multiple components are brought together for subsequent interaction at the molecular level.

The sole teaching of this reference with respect to ITP, then, is that it can be can be used as one way to combine assay components in a channel in high concentration, to facilitate reaction of the components with each other. This would not be perceived as a

useful benefit per se in a process in which the ultimate goal is to separate all of the components from each other. There is no suggestion that the use of ITP stacking would produce any particular advantage with respect to separation.

In particular, none of the references, alone or in combination, suggest the benefits of ITP stacking discovered by the Applicants, where the use of sample stacking in combination with a simple two-electrode injection scheme in a microchannel device produced separation results superior to the "pullback" and "pinching" strategies described in the prior art.

G. Claim 13 was rejected under 35 U.S.C. §103(a) as being unpatentable over Shultz-Lockyear *et al.* and Krivankova *et al.* in view of Fuchs *et al.* The rejections are respectfully traversed in light of the following remarks.

1. The Cited Art

SHULTZ-LOCKYEAR *ET AL.* is discussed above.

KRIVANKOVA *ET AL.* is discussed above.

FUCHS *ET AL.* is discussed above.

2. Analysis

As described above, the combination of Shultz-Lockyear *et al.* in view of Krivankova *et al.* does not teach or suggest the present method. Nor does Fuchs *et al.* make up for the deficiencies of this combination of references. As noted above, Fuchs *et al.* does not suggest the advantages of ITP in a separation method.

Accordingly, claim 13 is nonobvious over this combination of references. Therefore, Applicants respectfully request the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

H. Claims 1 and 13 was rejected under 35 U.S.C. §103(a) as being unpatentable over Fuchs *et al.* in view of Krivankova *et al.* and either Manz *et al.* or Shultz-Lockyear *et al.* The rejections are respectfully traversed in light of the following remarks.

1. The Cited Art

FUCHS *ET AL.* is discussed above.

KRIVANKOVA *ET AL.* is discussed above.

MANZ *ET AL.* is discussed above.

SHULTZ-LOCKYEAR *ET AL.* is discussed above.

2. Analysis

The deficiencies of the combination of Fuchs *et al.* with Krivankova *et al.*, and either of Manz *et al.* or Shultz-Lockyear *et al.* is addressed above. Briefly, the combinations of references fail to show or suggest motivation to combine the references along the lines of the present method and further fail to addressed the unsuggested results obtained with the present method.

In view of the foregoing, Applicants respectfully request the Examiner to withdraw the rejections under 35 U.S.C. §103(a).

II. Conclusion

In view of the foregoing, Applicants submit that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4410.

Respectfully submitted,

Date: October 30, 2006

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